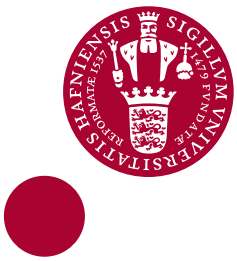

High-level spontaneous resistance to class IIA bacteriocins is developed by one general mechanism in Listeria Monocytogenes

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P008

THE *MYCOBACTERIUM TUBERCULOSIS* ESAT-6 GENE CLUSTERS: HIGH G+C GRAM-POSITIVE-SPECIFIC OPERONS THAT ARE FOUND IN MULTIPLE COPIES ONLY IN THE MYCOBACTERIA.

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The sequenced genome of *Mycobacterium tuberculosis* H37Rv revealed a region (the ESAT-6 gene cluster region), which is duplicated 5 times and encode several proteins of immunological importance. The genes present within this region include members of the ESAT-6 and CFP-10 families (both of which encode potent T-cell antigens of unknown function that are secreted without ordinary *sec*-dependant secretion signals), members of the PE and PPE families (multigene families proposed to be involved in antigenic variation) and the mycosins (secreted, membrane anchored, cell wall associated, subtilisin-like serine proteases). Other genes in this region include energy-providing ATPases as well as additional genes proposed to be involved in protein secretion. These gene clusters consist of all the necessary genes to form a dedicated, multi-component, binding protein-dependant active transport and processing system. It may thus be responsible for the *sec*-independent secretion of the ESAT-6 and CFP-10 family members. Several species of Gram-positive bacteria biosynthesize, process, and transport small, gene-encoded antimicrobial peptides (also lacking ordinary *sec*-dependant secretion signals) using a secretion system transcribed by genes organized in operons. This also includes genes encoding modification enzymes, dedicated subtilisin-like serine proteases, and ABC transporters. Thus, the *M. tuberculosis* ESAT-6 gene clusters contain some features of these operons. We have investigated the genomic organization, distribution, duplication, and localization of specific genes from these clusters and their potential contribution to the clinical manifestation of disease. The multiple duplications of the ESAT-6 gene cluster found in *M. tuberculosis* H37Rv were also found to be present in varying numbers in the genomes of other mycobacteria, e.g. *M. tuberculosis* strain 210 (5 copies), *M. tuberculosis* CDC1551 (5 copies), *M. bovis* (5 copies), *M. leprae* (2 functional copies), *M. avium* (4 copies), *M. paratuberculosis* (4 copies) and the avirulent *M. smegmatis* (3 copies). Phylogenetic analyses demonstrated that gene cluster region 4 is the ancestral region from which subsequent duplications have arisen and is also the only region of which an orthologue could be identified in each of the genomes of *Corynebacterium diptheriae* and *Streptomyces coelicolor*. Thus, comparative genomic analyses have revealed that the presence of the ESAT-6 gene cluster seems to be a characteristic exclusively shared by the high G+C gram-positive bacteria and that multiple duplications of this cluster have occurred and are maintained only in the genomes of members of the genus Mycobacterium. RT-PCR analyses were performed to strengthen the hypothesis that these gene clusters encode proteins involved in an ESAT-6 transport and processing system in the mycobacteria. The results demonstrate that ESAT-6 gene cluster region 3 is transcribed as a single polycistronic mRNA and that the other regions are also likely to be transcribed as one or more operons, indicating a shared function between the proteins. Studies have also been designed to confirm the secretion of ESAT-6 and CFP-10 by the gene cluster-encoded transport system at protein level. The multiplicity in the mycobacteria, the similarities to operons encoding antimicrobial peptides and cell-signaling molecules (which are potential virulence factors) as well as the fact that they contain several genes encoding proteins that are important in T-cell activation (a key factor in immunity and disease) suggest that the ESAT-6 gene clusters have an essential function in *M. tuberculosis* and could play a role in the development of disease.

P009

HIGH-LEVEL SPONTANEOUS RESISTANCE TO CLASS IIA BACTERIOCINS IS DEVELOPED BY ONE GENERAL MECHANISM IN *LISTERIA MONOCYTOGENES*.

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Class IIa bacteriocins may be used as natural food preservatives, yet resistance development in the target organisms is still poorly understood. In this study we continue the characterisation of class IIa resistance development in *Listeria monocytogenes*, linking the previous, seemingly contradictory results. Eight independent resistant mutants having a high resistance level (approx. 10^4-fold increase in MIC), originating from five wild-type listerial strains, were isolated following exposure to four different class IIa bacteriocin-producing lactic acid bacteria (including pediocin PA-1 and leucocin A producers). Two of the mutants were isolated from food model systems (a Saveloy-type sausage at 10°C, and salmon juice at 5°C). The eight mutants all had the previously observed increase in expression of a putative β-glucoside-specific PTS system (Gravesen *et al.*, 2000). However, disruption of this system in a resistant mutant did not confer pediocin sensitivity, indicating an indirect effect. The protein profiles of the mutant and wild-type strains were compared by 2D gel electrophoresis (see abstract by Ramnath *et al.*), and only one protein consistently disappeared following resistance development. Preliminary analysis indicates that this protein could be the mannose PTS enzyme EIIAB previously reported (Ramnath *et al.*, 2000). Inactivation of the mannose PTS system conferred class IIa resistance (Dalet *et al.*, accepted for publication), and these defined mutants also exhibited the two diverging PTS expression changes. The results indicate that high-level class IIa resistance in *L. monocytogenes* probably is developed by one prevalent mechanism, irrespective of wild-type strain, class IIa

bacteriocin, media, or environmental conditions. This mechanism is associated with the two different changes in PTS expression, where the β-glucoside PTS up-regulation is a secondary effect, whereas the mannose PTS may have a more direct involvement.

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P010

RAPID ENUMERATION AND IDENTIFICATION OF COLIFORMS AND *ESCHERICHIA COLI* USING A MODIFIED MPN TECHNIQUE.

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The issue of rapid microbiological identification is becoming increasingly important to be able to provide safe pharmaceuticals and food for the earth's growing population. In order to facilitate this requirement a rapid, sensitive test to enumerate and identify, simultaneously, total microbial numbers, coliforms and *Escherichia coli*, was developed. The test is based on the assumption that ß-D-galactosidase and ß-D-glucoronidase are markers for coliforms and *E.coli*, respectively and are not metabolized by non-coliform bacteria. Enumeration of all of the organisms is achieved using a MPN test with the milk to be tested used as the medium for growth, and a Tetrazolium salt reduction reaction, for enumeration. Coliforms and *E.coli* were identified on the basis of a further colour reaction using X-gal and *PNPG*, respectively. The test allowed enumeration as well as the identification of organisms within 4 hours.

P011

THE ROLE OF MYCOTHIOL IN THE SURVIVAL AND ADAPTIVE RESPONSE TO OXIDATIVE STRESS IN MYCOBACTERIA.

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One of the biggest concerns for the treatment of TB patients is the emergence of drug resistant strains of *Mycobacterium tuberculosis*, which makes treatment a longer and more expensive process and is not successful in all cases. Although specific mutations in genes have been identified which may be involved in rendering *M.tuberculosis* resistant to presently used drugs, not all resistance can be ascribed to these gene mutations and, therefore, there is a need to investigate other possible mechanisms utilized by *M.tuberculosis* in the detoxification of antimycobacterial drugs and mutagens. Mycothiol (MSH) is the major thiol present in the Actinomycetes and has been found highly expressed in *Mycobacterium smegmatis* and the important human pathogen, *M.tuberculosis*. These bacteria do not produce Glutathione (GSH), the major thiol involved in reactive oxygen species (ROS) detoxification found in the majority of eukaryotic and some prokaryotic organisms. MSH is considered to play a role analogous to that of GSH as an antioxidant that protects the cell against toxic oxygen stress. MSH is responsible for the detoxification of reactive oxygen species and alkylating agents via the MSH-dependant detoxification pathway. It is furthermore hypothesised that MSH may play a role in the detoxification of antibiotics, of which the major antituberculosis agent, Isoniazid, is one. The genes implicated in the synthesis of MSH, as well as those implicated in the MSH-dependant detoxification pathway, have been identified in *M. smegmatis* and *M. tuberculosis*. Using radioactive hybridisation techniques, we have shown that the genomic domains containing the genes have remained remarkably conserved in a diverse array of unrelated *M. tuberculosis* isolates and drug resistant isolates. This implies that these sequences have remained genetically unchanged throughout the evolutionary history of *M. tuberculosis*. Through quantitative RT-PCR, the activity of these genes in various stages of the bacterial growth cycle were evaluated. The genes implicated in MSH biosynthesis (MshB [Rv1170]) and reduction (Mycothione Reductase [Rv2855]) were shown to be transcribed in early log growth phase cultures, while none of the MSH- associated genes are transcribed in early stationary phase cultures (100 days). However in very late stationary phase, the gene involved in MSH biosynthesis were shown to be transcribed. These results indicate that MSH is required in the active phase of bacterial growth, possibly as a defence mechanism against intra- and extra cellular stress-inducing factors, but is suppressed in the early non-replicating phase of growth. The early logarithmic phase is an active growth phase where the most cellular ROS is produced and additional detoxification systems may be activated to help manage intracellular oxidative homeostasis. The up-regulation of MSH expression is observed in the far stationary phase, characterised by a downregulation of the majority of cellular activities and lack of nutrients, indicating that the mycobacterium may need to keep a basic detoxification process intact for survival. The above observations lend support to MSH as an antioxidant and shows that it may play an integral part in the growth and survival of *M.tuberculosis* during stressful conditions.

P012

THE GENETIC CONTRIBUTION TO TUBERCULOSIS SUSCEPTIBILITY.

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The Western Cape, South Africa, has one of the highest incidences of tuberculosis in the world, and the disease has reached epidemic proportions (up to 900/100 000) in the absence of any significant level of